PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

D3

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/12, 15/19, 15/57, 15/62, C07K 16/18, 16/28, 16/24, 16/40, C12Q 1/68, G01N 33/566, 33/68, A61K 38/17, 38/19, 38/48

A2

(11) International Publication Number: **WO 00/01817**

(43) International Publication Date: 13 January 2000 (13.01.00)

(21) International Application Number: PCT/US99/12366

(22) International Filing Date: 6 July 1999 (06.07.99)

(30) Priority Data:

09/110,938	6 July 1998 (06.07.98)	US
09/114,466	13 July 1998 (13.07.98)	US
60/093,897	23 July 1998 (23.07.98)	US
09/132,968	12 August 1998 (12.08.98)	US
09/136,214	18 August 1998 (18.08.98)	US
60/099,999	11 September 1998 (11.09.98)	US

(71) Applicant: SCHERING CORPORATION [US/US]; 2000 Galloping Hill Road, Kenilworth, NJ 07033–0530 (US).

(72) Inventors: BATES, Elizabeth, Esther, Mary; 4, place Gabriel Rambaud, F–69001 Lyon (FR). LEBECQUE, Serge, J., E.; 514, Chemin du Marand, F–69380 Civrieux d'Azergue (FR). MURPHY, Erin, E.; 180 Emerson Street, Palo Alto, CA 94301 (US). MATTSON, Jeanine, D.; 559 Alvarado Street, San Francisco, CA 94114 (US). GORMAN, Daniel, M.; 6371 Central Avenue, Newark, CA 94560 (US). HEDRICK, Joseph, A.; 52–08 Quail Ridge Drive, Plainsboro, NJ 08536 (US). WANG, Luquan; 21 Hollis Road, East Brunswick, NJ 08816 (US). ZLOTNIK, Albert; 507 Alger Drive, Palo Alto, CA 94306 (US). MURGOLO, Nicholas, J.; 99 Rolling Hill Drive, Millington, NJ 07946 (US). GREENE, Jonathan, R.; 457 Tillou Road, South Orange, NJ 07079 (US). JOHNSTON, James, A.; 205 Mary Alice Drive, Los Gatos, CA 95032 (US). BAZAN, Jose, Fernando; 775 University Drive, Menlo Park, CA 94025 (US). MAHONY, Daniel; 330 East 39th Street #21–A, New York, NY 10016 (US). LEES, Emma, M.; 3107 Washington Street, San Francisco, CA 94115 (US).

(74) Agents: THAMPOE, Immac, J. et al.; Schering–Plough Corporation, Patent Dept., K–6–1 1990, 2000 Galloping Hill Road, Kenilworth, NJ 07033–0530 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LU, LV, MD, MG, MK, MN, MX, NO, NZ, PL, PT, RO, RU, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: MAMMALIAN GENES; DENDRITIC CELL PROSTAGLANDIN–LIKE TRANSPONDER (DC–PGT), HDTEA84, HSLJD37R AND RANKL, HCC5 CHEMOKINE, DEUBIQUITINATING 11 AND 12 (DUB11, DUB12), MD–1, MD2 AND CYCLIN E2, RELATED REAGENTS AND METHODS

(57) Abstract

Purified genes from a mammal, reagents related thereto including purified proteins, specific antibodies, and nucleic acids encoding the polypeptides are provided. Methods of using said reagents and diagnostic kits are also provided. Characterization of genes and products relating to DC–PGT (Dendritic cell prostaglandin–like transporter), HDTEA84, HSLJD37R and RANKL (related to TNF receptor family), HCC5 chemokine, Dub 11 and Dub 12 (Deubiquitinating 11 and 12), MD–1 and MD–2 (proteins which exhibit properties of ligands for proteins exhibiting a leucine–rich protein motif (LRR)) and cyclin E2.

provides compositions which will be important in the control of cell division and transcription.

SUMMARY OF THE INVENTION

The present invention is based, in part, upon the characterization of the genes and products relating to the DC-PGT, HDTEA84, HSLJD37R, RANKL, HCC5 chemokine, Dub11, Dub12, MD-1, MD-2, and cyclin E2. It provides nucleic acids, polypeptides, antibodies, and methods for making and using such compositions.

In the DC-PGT embodiments, the invention provides an isolated or recombinant antigenic polypeptide comprising: a plurality of distinct segments, wherein each segment has identity to at least 12 contiguous amino acids from the mature SEQ ID NO: 2; or at least 17 contiguous amino acids from the mature SEQ ID NO: 2. In certain embodiments, the plurality of segments includes one of at least 19 contiguous amino acids; or two of at least 15 contiguous amino acids. Other polypeptides include those wherein the polypeptide: comprises the mature SEQ ID NO: 2; binds with specificity to a polyclonal antibody which specifically binds to SEQ ID NO: 2; or the polypeptide: is a natural allelic variant of SEQ ID NO: 2; is at least 30 amino acids in length; exhibits at least two non-overlapping epitopes specific for SEQ ID NO: 2; is a synthetic polypeptide; is attached to a solid substrate; or is a 5-fold or less conservative substitution from SEQ ID NO: 2. Fusion polypeptides are also provided, e.g., comprising first and second portions, the first portion comprising a sequence as described and the second portion comprising a detectable marker. Pharmaceutical compositions are made available, e.g., comprising a sterile polypeptide, as described, in a pharmaceutically acceptable carrier.

Polynucleotide embodiments include an isolated or recombinant polynucleotide encoding a described polypeptide. Preferred forms will be such a polynucleotide which: comprises the mature polypeptide coding portion of SEQ ID NO: 1; or encodes the mature SEQ ID NO: 2. Preferred embodiments include wherein the polynucleotide is: a PCR product; a hybridization probe; a

binds to a described polypeptide, comprising: incubating components comprising the compound and the polypeptide under conditions sufficient to allow the components to interact; and measuring the binding of the compound to the polypeptide.

In TNF receptor-like embodiments, the invention further provides an isolated or recombinant polynucleotide encoding an antigenic polypeptide comprising at least 17 contiguous amino acids from: the mature polypeptide from SEQ ID NO: 6; the mature polypeptide from SEQ ID NO: 8; the mature polypeptide from SEQ ID NO: 10; the mature polypeptide from SEQ ID NO: 12; the mature polypeptide from SEQ ID NO: 17; the mature polypeptide from SEQ ID NO: 19; the mature polypeptide from SEQ ID NO: 21; or the mature polypeptide from SEQ ID NO: 23. In preferred embodiments, such polynucleotide will encode all of the polypeptide of: signal processed SEQ ID NO: 6; signal processed SEQ ID NO: 8; signal processed SEQ ID NO: 10; signal processed SEQ ID NO: 12; signal processed SEQ ID NO: 17; SEQ ID NO: 19; SEQ ID NO: 21; or SEQ ID NO: 23. Other embodiments include such a polynucleotide, which hybridizes at 55° C, less than 500 mM salt, and 50% formamide to the: mature protein coding portion of SEQ ID NO: 5; signal processed coding portion of SEQ ID NO: 7; signal processed coding portion of SEQ ID NO: 9; signal processed coding portion of SEQ ID NO: 11; mature protein coding portion of SEQ ID NO: 16; polypeptide coding portion of SEQ ID NO: 18; polypeptide coding portion of SEQ ID NO: 20; or polypeptide coding portion of SEQ ID NO: 22. Other forms include those polynucleotides, comprising at least 35 contiguous nucleotides of: mature protein coding portion of SEQ ID NO: 5; signal processed coding portion of SEQ ID NO: 7; signal processed coding portion of SEQ ID NO: 9; signal processed coding portion of SEQ ID NO: 11; mature protein coding portion of SEQ ID NO: 16; polypeptide coding portion of SEQ ID NO: 18; polypeptide coding portion of SEQ ID NO: 20; or polypeptide coding portion of SEQ ID NO: 22. Various expression vectors are provided comprising such a polynucleotide. The invention also provides a host cell containing the expression vector, including a eukaryotic cell.

sample is from a human, and the binding compound is an antibody. Such also allow for production of a detection kit comprising the binding compound, and: instructional material for the use of the binding compound for the detection; or a compartment providing segregation of the binding compound.

Polypeptides are also made available, e.g., a substantially pure or isolated antigenic polypeptide, which binds to the described binding composition, and further comprises at least 17 contiguous amino acids from: signal processed SEQ ID NO: 6; signal processed SEQ ID NO: 8; signal processed SEQ ID NO: 10; signal processed SEQ ID NO: 12; signal processed SEQ ID NO: 17; SEQ ID NO: 19; SEQ ID NO: 21; or SEQ ID NO: 23. Preferred polypeptides include those which: comprise at least a fragment of at least 25 contiguous amino acid residues from: a primate HDTEA84 protein; a primate HSLJD37R protein; or a rodent or primate RANKL protein; or are soluble polypeptides; are detectably labeled; are in a sterile composition; are in a buffered composition; bind to an sialic acid residue; are recombinantly produced; or have a naturally occurring polypeptide sequence. In other embodiments, the polypeptide comprises at least 17 contiguous amino acids from the: signal processed SEQ ID NO: 6; signal processed SEQ ID NO: 8; signal processed SEQ ID NO: 12; signal processed SEQ ID NO: 17; SEQ ID NO: 19; SEQ ID NO: 21; or SEQ ID NO: 23.

Methods are provided, including a method of modulating a precursor cell physiology or function comprising a step of contacting the cell with: a binding compound which binds to a described polypeptide; an HDTEA84 polypeptide; an HSLJD37R polypeptide; or a RANKL polypeptide. The method may be one wherein the contacting is in combination with a TNF family ligand, or an antagonist of the TNF family ligand.

In other embodiments, the present invention provides compositions related to other chemokine, Dub, or surface protein genes. Polypeptide embodiments include: a substantially pure or recombinant HCC5 polypeptide exhibiting identity over a length of at least 12 amino acids to SEQ ID NO: 25; an isolated natural sequence HCC5 of mature SEQ ID NO: 25; a fusion protein comprising HCC5 sequence; a substantially pure or recombinant Dub11

21. A method of detecting the presence of a complementary polynucleotide in a sample, comprising contacting a polynucleotide of Claim 6 that selectively hybridizes with said complementary polynucleotide in said sample to form a detectable duplex; thereby indicating the presence of said polynucleotide in said sample.

22. A method for identifying a compound that binds to a polypeptide of Claim 1, comprising:

a) incubating components comprising said compound and said polypeptide under conditions sufficient to allow the components to interact; and

b) measuring the binding of the compound to said polypeptide.

23. An isolated or recombinant polynucleotide encoding an antigenic polypeptide comprising:

a) at least 17 contiguous amino acids from the mature polypeptide from SEQ ID NO: 6;

b) at least 17 contiguous amino acids from the mature polypeptide from SEQ ID NO: 8;

c) at least 17 contiguous amino acids from the mature polypeptide from SEQ ID NO: 10;

d) at least 17 contiguous amino acids from the mature polypeptide from SEQ ID NO: 12;

e) at least 17 contiguous amino acids from the mature polypeptide from SEQ ID NO: 17;

f) at least 17 contiguous amino acids from the mature polypeptide from SEQ ID NO: 19;

g) at least 17 contiguous amino acids from the mature polypeptide from SEQ ID NO: 21; or

h) at least 17 contiguous amino acids from the mature polypeptide from SEQ ID NO: 23.

24. The polynucleotide of Claim 23, encoding all of the polypeptide of:

a) signal processed SEQ ID NO: 6;

b) signal processed SEQ ID NO: 8;

c) signal processed SEQ ID NO: 10;

d) signal processed SEQ ID NO: 12;
e) signal processed SEQ ID NO: 17;
f) SEQ ID NO: 19;
g) SEQ ID NO: 21; or
h) SEQ ID NO: 23.

25. The polynucleotide of Claim 23, which hybridizes at 55° C, less than 500 mM salt, and 50% formamide to the:
a) mature protein coding portion of SEQ ID NO: 5;
b) signal processed coding portion of SEQ ID NO: 7;
c) signal processed coding portion of SEQ ID NO: 9;
d) signal processed coding portion of SEQ ID NO: 11;
e) mature protein coding portion of SEQ ID NO: 16;
f) polypeptide coding portion of SEQ ID NO: 18;
g) polypeptide coding portion of SEQ ID NO: 20; or
h) polypeptide coding portion of SEQ ID NO: 22.

26. The polynucleotide of Claim 25, comprising at least 35 contiguous nucleotides of:
a) mature protein coding portion of SEQ ID NO: 5;
b) signal processed coding portion of SEQ ID NO: 7;
c) signal processed coding portion of SEQ ID NO: 9;
d) signal processed coding portion of SEQ ID NO: 11;
e) mature protein coding portion of SEQ ID NO: 16;
f) polypeptide coding portion of SEQ ID NO: 18;
g) polypeptide coding portion of SEQ ID NO: 20; or
h) polypeptide coding portion of SEQ ID NO: 22.

27. An expression vector comprising the polynucleotide of Claim 23.

28. A host cell containing the expression vector of Claim 27, including a eukaryotic cell.

29. A method of making an antigenic polypeptide comprising expressing a recombinant polynucleotide of Claim 23.

30. A method for detecting a polynucleotide of Claim 23, comprising contacting said polynucleotide with a probe that hybridizes, under stringent conditions, to at least 25 contiguous nucleotides of the:

a) mature protein coding portion of SEQ ID NO: 5;
b) signal processed coding portion of SEQ ID NO: 7;
c) signal processed coding portion of SEQ ID NO: 9;
d) signal processed coding portion of SEQ ID NO: 11;
e) mature protein coding portion of SEQ ID NO: 16;
f) polypeptide coding portion of SEQ ID NO: 18;
g) polypeptide coding portion of SEQ ID NO: 20; or
h) polypeptide coding portion of SEQ ID NO: 22;

to form a duplex, wherein detection of said duplex indicates the presence of said polynucleotide.

31. A kit for the detection of a polynucleotide of Claim 23, comprising a compartment containing a probe that hybridizes, under stringent hybridization conditions, to at least 17 contiguous nucleotides of a polynucleotide of Claim b1 to form a duplex.

32. The kit of Claim 31, wherein said probe is detectably labeled.

33. A binding compound comprising an antibody binding site which specifically binds to a polypeptide comprising at least 17 contiguous amino acids from:

a) signal processed SEQ ID NO: 6;
b) signal processed SEQ ID NO: 8;
c) signal processed SEQ ID NO: 10;
d) signal processed SEQ ID NO: 12;
e) signal processed SEQ ID NO: 17;
f) SEQ ID NO: 19;
g) SEQ ID NO: 21; or
h) SEQ ID NO: 23.

34. The binding compound of Claim 33, wherein:

a) said antibody binding site is:

1) selectively immunoreactive with the:
a) signal processed SEQ ID NO: 6;
b) signal processed SEQ ID NO: 8;
c) signal processed SEQ ID NO: 10;
d) signal processed SEQ ID NO: 12;
e) signal processed SEQ ID NO: 17;
f) SEQ ID NO: 19;
g) SEQ ID NO: 21; or
h) SEQ ID NO: 23;
2) raised against a purified or recombinantly produced human HDTEA84 protein;
3) raised against a purified or recombinantly produced human HSLJD37R protein; or
4) in a monoclonal antibody, Fab, or F(ab)2; or

b) said binding compound is:
1) an antibody molecule;
2) a polyclonal antiserum;
3) detectably labeled;
4) sterile; or
5) in a buffered composition.

35. A method using the binding compound of Claim 33, comprising contacting said binding compound with a biological sample comprising an antigen, thereby forming a binding compound:antigen complex.

36. The method of Claim 35, wherein said biological sample is from a human, and wherein said binding compound is an antibody.

37. A detection kit comprising said binding compound of Claim 34, and:
a) instructional material for the use of said binding compound for said detection; or
b) a compartment providing segregation of said binding compound.

38. A substantially pure or isolated antigenic polypeptide, which binds to said binding composition of Claim 33, and further comprises at least 17 contiguous amino acids from:

a) signal processed SEQ ID NO: 6;
b) signal processed SEQ ID NO: 8;
c) signal processed SEQ ID NO: 10;
d) signal processed SEQ ID NO: 12;
e) signal processed SEQ ID NO: 17;
f) SEQ ID NO: 19;
g) SEQ ID NO: 21; or
h) SEQ ID NO: 23.

39. The polypeptide of Claim 38, which:

a) comprises at least a fragment of at least 25 contiguous amino acid residues from a primate HDTEA84 protein;
b) comprises at least a fragment of at least 25 contiguous amino acid residues from a primate HSLJD37R protein;
c) comprises at least a fragment of at least 25 contiguous amino acid residues from a rodent or primate RANKL protein;
d) is a soluble polypeptide;
e) is detectably labeled;
f) is in a sterile composition;
g) is in a buffered composition;
h) binds to an sialic acid residue;
i) is recombinantly produced, or
j) has a naturally occurring polypeptide sequence.

40. The polypeptide of Claim 39, which comprises at least 17 contiguous amino acids from the:

a) signal processed SEQ ID NO: 6;
b) signal processed SEQ ID NO: 8;
c) signal processed SEQ ID NO: 10;
d) signal processed SEQ ID NO: 12;
e) signal processed SEQ ID NO: 17;
f) SEQ ID NO: 19;
g) SEQ ID NO: 21; or
h) SEQ ID NO: 23.

```
agt tgc atc acc tgt gct gtc atc aat cgt gtt cag aag gtc aac tgc   302
Ser Cys Ile Thr Cys Ala Val Ile Asn Arg Val Gln Lys Val Asn Cys
 60                  65                  70                  75

aca gct acc tct aat gct gtc tgt ggg gac tgt ttg ccc agg ttc tac   350
Thr Ala Thr Ser Asn Ala Val Cys Gly Asp Cys Leu Pro Arg Phe Tyr
                 80                  85                  90

cga aag aca cgc att gga ggc ctg cag gac caa gag tgc atc ccg tgc   398
Arg Lys Thr Arg Ile Gly Gly Leu Gln Asp Gln Glu Cys Ile Pro Cys
             95                 100                 105

acg aag cag acc ccc acc tct gag gtt caa tgt gcc ttc cag ttg agc   446
Thr Lys Gln Thr Pro Thr Ser Glu Val Gln Cys Ala Phe Gln Leu Ser
        110                 115                 120

tta gtg gag gca gat gca ccc aca gtg ccc cct cag gag gcc aca ctt   494
Leu Val Glu Ala Asp Ala Pro Thr Val Pro Pro Gln Glu Ala Thr Leu
    125                 130                 135

gtt gca ctg gtg agc agc ctg cta gtg gtg ttt acc ctg gcc ttc ctg   542
Val Ala Leu Val Ser Ser Leu Leu Val Val Phe Thr Leu Ala Phe Leu
140                 145                 150                 155

ggg ctc ttc ttc ctc tac tgc aag cag ttc ttc aac aga cat tgc cag   590
Gly Leu Phe Phe Leu Tyr Cys Lys Gln Phe Phe Asn Arg His Cys Gln
                160                 165                 170

cgt gga ggt ttg ctg cag ttt gag gct gat aaa aca gca aag gag gaa   638
Arg Gly Gly Leu Leu Gln Phe Glu Ala Asp Lys Thr Ala Lys Glu Glu
            175                 180                 185

tct ctc ttc ccc gtg cca ccc agc aag gag acc agt gct gag tcc caa   686
Ser Leu Phe Pro Val Pro Pro Ser Lys Glu Thr Ser Ala Glu Ser Gln
        190                 195                 200

gtc tct tgg gcc cct ggc agc ctt gcc cag ttg ttc tct ctg gac tct   734
Val Ser Trp Ala Pro Gly Ser Leu Ala Gln Leu Phe Ser Leu Asp Ser
    205                 210                 215

gtt cct ata cca caa cag cag cag ggg cct gaa atg tgatgtccac        780
Val Pro Ile Pro Gln Gln Gln Gln Gly Pro Glu Met
220                 225                 230

angagctaat accctacaga tggggcatat cctatcccat cccaccagag gattgattct 840

ccatttcaca aggactgatc tggagcattt cttgcttccc tgttgtagtc tggggagcca 900

gattccacat tcatgggact accagacatg tt                               932
```

<210> 23
<211> 231
<212> PRT
<213> Unknown

<400> 23

```
Met Asp Cys Gln Glu Asn Glu Tyr Trp Asp Gln Trp Gly Arg Cys Val
  1               5                  10                  15
```

Thr Cys Gln Arg Cys Gly Pro Gly Gln Glu Leu Ser Lys Asp Cys Gly
20 25 30

Tyr Gly Glu Gly Gly Asp Ala Tyr Cys Thr Ala Cys Pro Pro Arg Arg
35 40 45

Tyr Lys Ser Ser Trp Gly His His Lys Cys Gln Ser Cys Ile Thr Cys
50 55 60

Ala Val Ile Asn Arg Val Gln Lys Val Asn Cys Thr Ala Thr Ser Asn
65 70 75 80

Ala Val Cys Gly Asp Cys Leu Pro Arg Phe Tyr Arg Lys Thr Arg Ile
85 90 95

Gly Gly Leu Gln Asp Gln Glu Cys Ile Pro Cys Thr Lys Gln Thr Pro
100 105 110

Thr Ser Glu Val Gln Cys Ala Phe Gln Leu Ser Leu Val Glu Ala Asp
115 120 125

Ala Pro Thr Val Pro Pro Gln Glu Ala Thr Leu Val Ala Leu Val Ser
130 135 140

Ser Leu Leu Val Val Phe Thr Leu Ala Phe Leu Gly Leu Phe Phe Leu
145 150 155 160

Tyr Cys Lys Gln Phe Phe Asn Arg His Cys Gln Arg Gly Gly Leu Leu
165 170 175

Gln Phe Glu Ala Asp Lys Thr Ala Lys Glu Glu Ser Leu Phe Pro Val
180 185 190

Pro Pro Ser Lys Glu Thr Ser Ala Glu Ser Gln Val Ser Trp Ala Pro
195 200 205

Gly Ser Leu Ala Gln Leu Phe Ser Leu Asp Ser Val Pro Ile Pro Gln
210 215 220

Gln Gln Gln Gly Pro Glu Met
225 230

<210> 24
<211> 232
<212> DNA
<213> Unknown

<220>
<223> Description of Unknown Organism:primate

<400> 24
ttggccttag ggaccaagct tttatcatcg tcagtgggac ttaacctgtc ttaaaagtgc 60

tgcttctcct acactcgctc aagatcccga gtcagctgta ttatggcatc ctattagtca 120

ggcagcctgt gcttcaagcc cgtagttgta ttcatcccct aaaggggcca ttccgtttgt 180

atcatcacat gtcctcagtg ggtccatgtg tatatcaagg acatgatgca ga 232